FavorPrep™
Tissue Total RNA Mini Kit

User Manual

Cat. No.: FATRK 001 (50 Preps)
FATRK 001-1 (100 Preps)
FATRK 001-2 (300 Preps)

For Research Use Only

v.1304

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Introduction

FavorPrep Tissue Total RNA Extraction Mini Kit is desigened for extraction of total RNA from animal tissue and cultured cells. Some specially modified protocols are developed for other samples, such as bacteria and yeast. This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH2O. It takes 30 min for an entire procedure, and the pyrified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

Sample amount and yield

Sample	Recommended amount of sample used		Yield (μg)
Animal cells (up to 5 x 10^6)	NIH/3T3	1 x 10^6 cells	10
	HeLa	1 x 10^6 cells	15
	COS-7	1 x 10^6 cells	30
	LMH	1 x 10^6 cells	12
Animal tissues (Mouse/rat) (up to 30 mg)	Embryo	10 mg	25
	Heart	10 mg	10
	Brain	10 mg	10
	Kidney	10 mg	30
	Liver	10 mg	50
	Spleen	10 mg	35
	Lung	10 mg	15
	Thymus	10 mg	45
Bacteria	*E. coli*	1 x 10^9 cells	60
	B. subtilis	1 x 10^9 cells	40
Yeast (up to 5 x 10^7)	*S. cerevisiae*	1 x 10^7 cells	25

Handling time: about 30 min

Kit Contents

Cat. No. / preps	FATRK001 (50 preps)	FATRK001-1 (100 preps)	FATRK001-2 (300 preps)
FARB Buffer	25 ml	45 ml	130 ml
Wash Buffer 1	30 ml	60 ml	170 ml
Wash Buffer 2 (concentrated)	15 ml *	35 ml **	50 ml *** x 2
RNase-free ddH2O	6 ml	6 ml	8 ml X2
Filter Column	50 pcs	100 pcs	300 pcs
FARB Mini Column	50 pcs	100 pcs	300 pcs
Collection Tube	100 pcs	200 pcs	600 pcs
Micropestle	50 pcs	100 pcs	300 pcs
Elution Tube	50 pcs	100 pcs	300 pcs
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* Add 60 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
** Add 140 ml ethanol (96-100 %) to Wash Buffer 2 when first open.
*** Add 200 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

Important notes

1. Make sure everything is RNase-free when handling RNA.

2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling hese buffers.

3. Pipet a required volume of FARB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer before use.

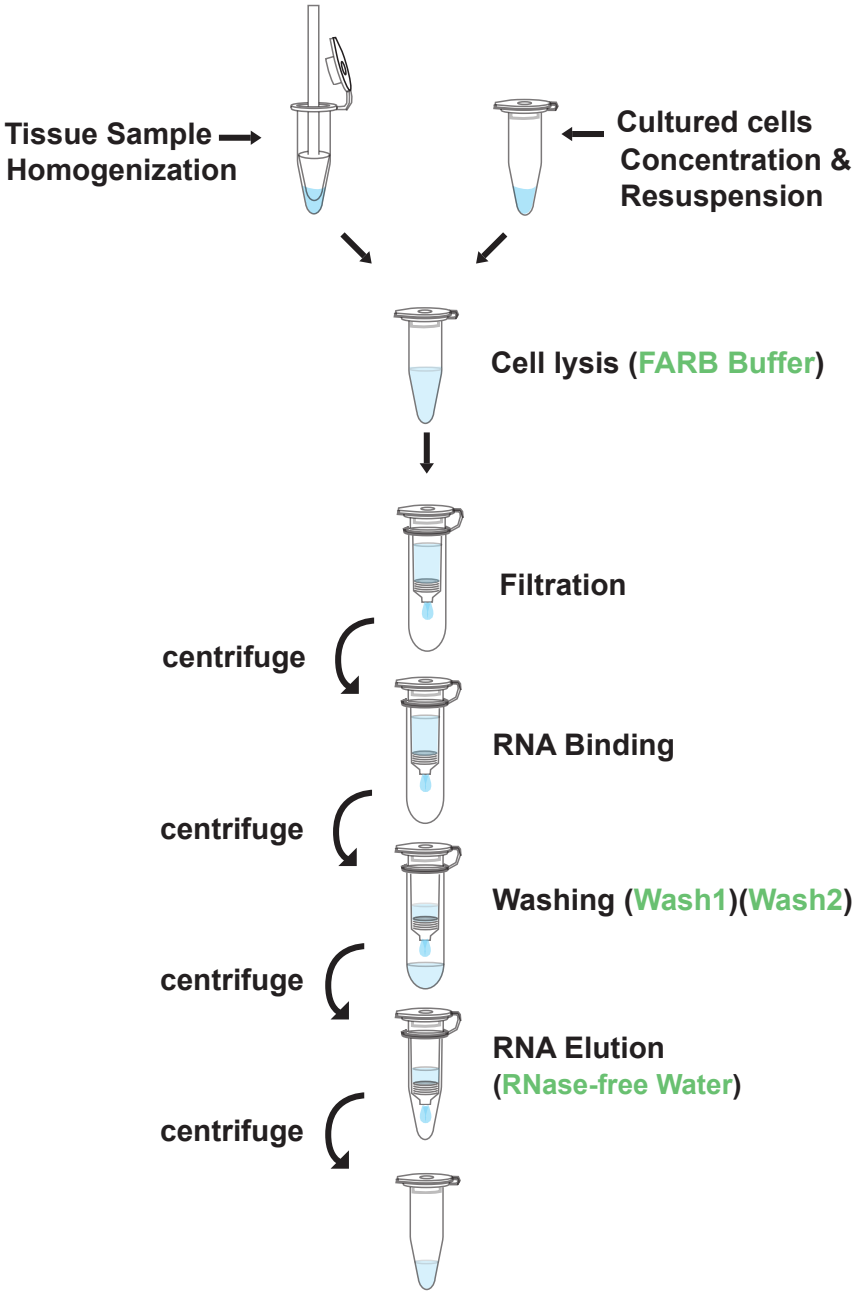
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 as bottle indicated when first open.

5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

6. Dilute RNase-free DNase 1 in reaction buffer (1M NaCl, 10mM $MnCl_2$, 20 mM Tris-HCl, pH 7.0 at 25°C) to final conc. = 0.5 U/µl.

7. The additional equipment, 20-G needle syringe, is needed for extraction of total RNA from tissue sample.

Brief Procedure



General Protocol: (For Animal Cells)

Please Read Important Notes Before Starting The Following Steps.

1. **Pellet 1 ~ 5 ×10^6 cells by centrifuge at 300 x g for 5 min. Remove all the supernatant.**

2. **Add 350 µl of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously to lyse the cells. Incbuate at room temperature for 5 min.**

> **Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.**

3. **Place a Filter Column into a Collection Tube and transfer the sample mixture to Filter Column, centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min.**

4. **Transfer the clarified supernatant from Collection Tube to a new micro-centrifuge tube (not provided) and adjust the volume of the clear lysate.**
   --Avoid pipetting any debris and pellet from Collection Tube.

5. **Add 1 volume of 70% ethanol to the clear lysate and mix well by vortexing.**

6. **Briefly spin the tube to remove drops from the inside of the lid.**
   Place a FARB Mini Column into a Collection, transfer the ethanol added sample (including any precipitate) to FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min and discard the flow-through.

7. **(Optional): To eliminate genomic DNA contamination, follow the steps from 7a. Otherwise, proceed to step 8 directly.**

   7a. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
   7b. Add 60 µl of RNase-free DNase 1 solution (0.5U/µl, not provided) to the membrane center of FARB Mini Column. Place the Column on the benchtop for 15 min.
   7c. Add 250 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
   7d. After DNase 1 treatment, proceed to step 9.

8. **Add 500 µl of Wash Buffer 1 to wash FARB Mini Column. Centrifugeat at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**

9. **Wash FARB Mini Column twice with 750 µl of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.**
   --Make sure that ethanol has been added into Wash Buffer 2 when first open.

10. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the column.**
    --**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

11. **Place FARB Mini Column to Elution Tube.**

12. **Add 50 µl of RNase-free ddH_2O to the membrane center of FARB Mini Column. Stand FARB Mini Column for 1 min.**
    --**Important Step!** For effective elution, make sure that RNase-free ddH_2O is dispensed on the membrane center and is absorbed completely.

13. **Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.**

14. **Store RNA at -70C.**

Special Protocol: (For Animal Tissue)

Additional equipment: a 20-G needle syringe

1. **(For Fresh sample): Cut up to 30 mg of tissue sample. Grind the tissue sample completely in liquid nitrogen. Transfer the powder to a new microcentrifuge tube(not provided). Or you can place tissue sample into a microcentrifuge tube and use provided micropestle to grind the tissue sample few times and break it into small pieces.**
   (For Frozen sample) Weight up to 30 mg tissue sample and grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube (not provided).

2. **Add 350 µl of FARB Buffer (ß-ME added) to the sample and shear this tissue sample by passing lysate through a 20-G needle syringe 10 times. Incubate at room temperature for 5 min.**
   --Grind the sample a few times to make it break more completely.

Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

3. **Follow the Animal Cells Protocol starting from step 3.**

Special Protocol: (For Bacteria)

1. Transfer 1 ml well-grown bacterial culture (or up to 1x10^9 cells) to a microcentrifuge tube (not provided).
2. Descend the bacterial cells by centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min and discard the supernatant completely.
3. Resuspend the cell pellet in 100 µl of RNase-free lysozyme reaction solution (20mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition) (not provided).
4. Incubate at 37°C for 10 min.
5. Add 350 µl of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incbuate at room temperature for 5 min.

Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

6. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insouble material and transfer the supernatant to a microcentrifge tube (not provided) and adjust the volume of the clear lysate.
   -Avoid pipetting any debris and pellet in the Collection Tube.
7. Follow the Animal Cells Protocol starting from step 5.

Special Protocol: (For Yeast)

1. Transfer 3 ml of log-phase (OD600=10) yeast culture to a microcentrifuge tube (not provided).
2. Descend the yeast cells by centrifug at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.
3. Resuspend the cell pellet in 600 µl sorbitol buffer (1 M sorbitol; 100 mM EDTA; 0.1% ß-ME) (not provided). Add 200 U zymolase or lyticase and incubate at 30 °C for 30 min.
   --Prepare sorbitol buffer just before use.
4. Centrifuge at 7,500 rpm (5,000xg) for 5 min to pellte the spheroplasts. Discard the supernatant.
5. Add 350 µl of FARB Buffer (ß-ME added) to the pellet and vortex vigorously to lyse the spheroplasts. Incbuate at room temperature for 5 min.

Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

6. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to spin down insoluble materials and transfer the supernatant to a microcentrifuge tube (not provided).
7. Follow the Animal Cells Protocol starting from step 5.

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Tissue Total RNA Maxi Kit

User Manual

Cat. No.: FATRK 003 (10 Preps)
FATRK 003-1 (24 Preps)

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Introduction

FavorPrep Tissue Total RNA Extraction Maxi Kit is desigened for extraction of total RNA from a variety of animal tissues and cells. Some specially modified protocols are developed for other samples, such as bacteria and yeast. This method first lyses cells by using a chaotropic salt , then binds RNA to silica-based membranes, washes RNA with ethanol-contained wash buffer and then elutes purified RNA by RNase-free ddH_2O. It takes 60 min for an entire procedure, and the purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library contruction.

Sample amount:

0.5 ~1 g of animal tissue

Up to 5 X 10^{10} bacteria cells

Up to 5 X 10^{9} yeast culture

Up to 5 ×10^{8} of animal cells

Handling time: about 60 min

Kit Contents

Cat. No. / preps	FATRK003 (10 preps)	FATRK003-1 (24 preps)
FARB Buffer	150 ml	180 ml X2
Wash Buffer 1	135 ml	160 ml X2
Wash Buffer 2 (concentrated)	27 ml X2	27 ml X5
RNase-free ddH_2O	12 ml	30 ml
Filter Column	10 pcs	24 pcs
FARB Maxi Column	10 pcs	24 pcs
Elution Tube (50 ml tube)	10 pcs	24 pcs
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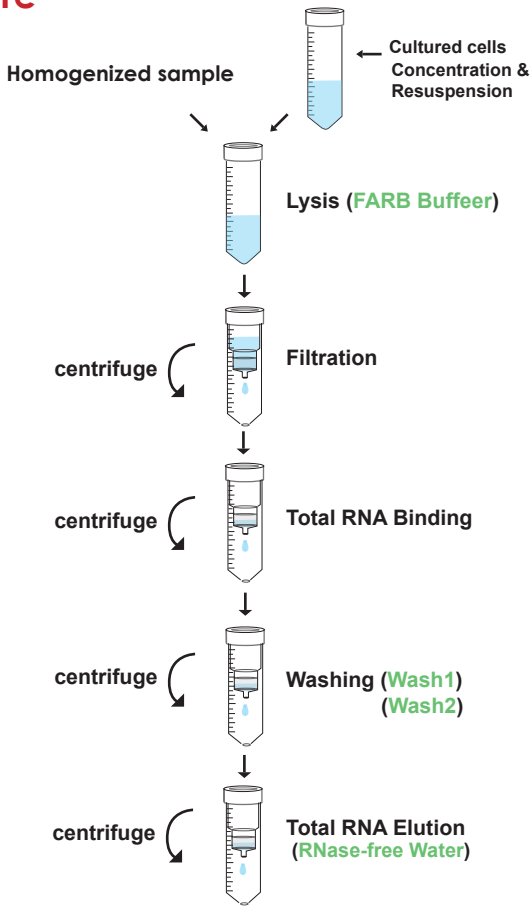
* Add 108 ml ethanol (96-100 %) to each Wash Buffer 2 when first open.

Important notes

1. Make sure everything is RNase-free when handling RNA.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling hese buffers.
3. Pipet a required volume of FARB Buffer to another RNase-free container and add 10 µl ß-mercaptoethanol (ß-ME) per 1ml FARB Buffer before use.
4. Add required volume of RNase-free ethanol (96~100%) to Wash Buffer 2 as bottle indicated when first open.
5. Dilute RNase-free DNase 1 in reaction buffer (150mM NaCl, 1 mM $MgCl_2$, 10 mM Tris HCl, pH 7.5) to final conc. = 2KU/ml. (1 ml /preparation)
6. Use a centrifuge with a swinging bucket rotor for 15ml (Midi) or 50ml (Maxi) in all centrifugation steps. The maximum speed should be 3500-5000 rpm or 3000-5000 x g.

Brief Procedure



Genernal Protocol: (For Animal Tissue)

Please Read Important Notes Before Starting The Following Steps.

Additional equipment: a 20-G needle syringe

1. **Cut off 0.5 g (up to 1 g) of tissue sample and grind the tissue sample completely under liquid nitrogen to a find powder then transfer the powder to a 50 ml centrifuge tube.**

> Note: Do not use to much sample in this RNA extraction procedure ! It is important to use the correct number of starting cells in order to obtain optimal RNA yield and purity.

2. **Add 14 ml of FARB Buffer (ß-ME added) to the sample and shear this tissue sample by passing lysate through a 20-G needle syringe 10 times**

> Note: In order to release all the RNA in the sample, it is required to disrupt the sample completely. Different samples require different methods (ex: disruptor equipment) to achieve complete disruption.

3. **Incubate the sample mixture at room temperture for 5 minutes.**

4. **Place a Filter Maxi Column in a clean 50 ml tube (not provided), and transfer the sample mixture to Filter Maxi Column, centrifuge at full speed for 5 min.**

5. **Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided), and adjust the volume of the clear lysate.**
   --Avoid to disrupt any debris and pellet when transfer the supernatant.

6. **Add an equal volume of 70% ethanol to the clear lysate and mix well by vortexing.**

7. **Place a FARB Maxi Column in a clean 50 ml tube (not provided), and transfer 14 ml of the ethanol added sample (including any precipitate) to FARB Maxi Colum. Centrifuge at full speed for 5 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube. Then repeat this step for the rest sample mixture.**

8.(Optional): To eliminate genomic DNA contamination, follow the steps from 8a. Otherwise, proceed to step 9 directly.

8a. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.

8b. Add 0.5 ml of RNase-free DNase 1 solution (2U/µl, not provided) to the membrane center of FARB Maxi Column. Place the Column on the benchtop for 10 min.

8c. Add 7 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.

8d. After DNase 1 treatment, proceed to step 10.

9. Add 12.5 ml of Wash Buffer 1 to wash FARB Maxi Column, centrifuge at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.

10. Wash FARB Maxi Column twice with 12.5 ml of Wash Buffer 2 by centrifuging at full speed for 2 min. Discard the flow-through and place the FARB Maxi Column back in 50 ml centrifuge Tube.
--Make sure that ethanol has been added into Wash Buffer 2 when first open.

11. Centrifuge at full speed (> 4,000 x g) for an additional 10 min to dry the FARB Maxi Column.
--**Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

12. Place FARB Maxi Column in Elution Tube (50 ml tube, provided).

13. Add 500~1000 µl of RNase-free Water to the membrane center of FARB Maxi Column. Stand FARB Maxi Column for 5 min.
--**Important Step!** For effective elution, make sure that RNase-free Water is dispensed on the membrane center and is absorbed completely.

14. Centrifuge at full speed for 5 min to elute RNA.

15. Store RNA at -70ºC.

Special Protocol: (For Animal Cells)

1. **Pellet Up to 5 ×10^8 of animal cells by centrifuge at 300 x g for 5 min. Discard the supernatant completely.**
2. **Add 14 ml of FARB Buffer (ß-ME added) to the cell pellet and vortex vigorously. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
3. **Place a Filter Maxi Column in a 50 ml tube (not provided), and transfer the sample mixture to Filter Maxi Column, centrifuge at full speed for 5 min.**
4. **Transfer the clarified supernatant from previous step to a clean 50 ml tube (not provided) and adjust the volume of the clear lysate.**
   --Avoid pipetting any debris and pellet from this Collection Tube.
5. **Add an equal volume of 70% ethanol to the clear lysate and mix well by pipetting.**
6. **Follow the General Protocol starting from step 7.**

Special Protocol: (For Bacteria)

1. **Transfer Up to 5 X 10^{10} of well-grown bacterial to a entrifuge tube (not provided).**
2. **Descend the bacterial cells by centrifuge at >3,000 x g for 5 min and discard the supernatant completely.**
3. **Resuspend the cell pellet in 1 ml of RNase-free lysozyme reaction solution (20mg/ml lysozyme; 20mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2% Trition) (not provided).**
4. **Incubate at 37°C for 10 min.**
5. **Add 14 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortex. Incubate at room temperature for 5 min.**
   (For preparation of FARB Buffer (ß-ME added), see Important Note: 3)
6. **Centrifuge at full speed for 5 min to spin down insouble material and transfer the supernatant to a 50 ml tube. (not provided)**
7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**
8. **Follow the General Protocol starting from step 7.**

Special Protocol: (For Yeast)

1. **Transfer up to 5 X 10^9 ml of log-phase (OD600=10) yeast culture to a 50 ml centrifuge tube. (not provided)**
2. **Descend the yeast cells by centrifug at 500 x g at 4 °C for 5 min and discard the supernatant completely.**
3. **Resuspend the cell pellet in 2.5 ml of enzymatic lysis buffer (20 mg/ml lyticase or zymolase; 1M sorbitol; 100mM EDTA; 0.1% ß-ME) (not provided). And incubate at 30 °C for 30 min.**
   --Prepare sorbitol buffer just before use.
4. **Centrifuge at 500 x g at room temperature for 5 min to pellet spheroplasts and discard the supernatant completely.**
5. **Add 14 ml of FARB Buffer (ß-ME added) to the sample and mix well by vortexing. Incubate at room temperature for 5 minutes.**
6. **Centrifuge at full speed for 5 min to spin down insoluble materials and transfer the clarified supernatant to a 50 ml tube (not provided).**
7. **Add an equal volume of 70% ethanol to the clear lysate and mix by pipetting.**
8. **Follow the General Protocol starting from step 7.**